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Relation	

Title

Thermosensitive properties of semi-IPN gel composed of amphiphilic gel and zwitterionic thermosensitive polymer in buffer solutions containing high concentration salt

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Abstract

In this study, a semi-interpenetrating polymer network (semi-IPN) gel, which consists of an amphiphilic *N*,*N*-dimethylacrylamide-*co*-*N*-isopropylacrylamide (DMAA-*co*-NIPAM) gel and an interpenetrating zwitterionic thermosensitive poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethyl(acrylamidopropyl)ammonium propane sulfonate) (poly(NIPAM-*co*-DMAAPS)) was prepared. The thermosensitive behavior of the semi-IPN gel was investigated in a buffer solution composed of a relatively high concentration of sodium chloride and sodium citrate as salts, and sodium dodecyl sulfate as a surfactant, which are generally used as a buffer solution in biochips. At low temperatures, the semi-IPN gel in the buffer solution was absolutely transparent; however, when the gel was heated, the gel became milky white or opaque without a large change in the gel size. The network of the transparent gel is homogeneous, whereas that of the opaque gel consists of coarse and dense parts. Such a structural change in the gel network was confirmed by the temperature dependence of the permeability of the buffer solution through the semi-IPN gel membrane. The permeability increased drastically when the gel became opaque because of heating.

Key Words: Semi-IPN gel; Zwitterionic polymer; Thermosensitive property

1. Introduction

Recently, the application of polymeric hydrogels to biochips such as DNA chips and protein chips has attracted special attention in the field of biotechnology [1-5]. In hydrogel-type biochips, the capture probes are immobilized on the gel network, and the target probes diffuse through the gel network and react with the capture probes. The hydrogel-type biochip offers the advantage of having a larger immobilization capacity than that of conventional biochips, in which the capture probes are immobilized two-dimensionally on a flat plate. Furthermore, in the hydrogel-type biochip, the gel network, which serves as a matrix that holds water, prevents the chips from drying.

The important requirements for the hydrogel-type biochip are as follows:

(a) High diffusivity or permeability of the capture probes such as DNA through the gel network in the reaction process. The reaction is carried out at relatively high temperature, i.e. 50 to 70°C.

(b) High transparency of the gel at room temperature. The hybridization reaction is usually detected by means of a fluorescence technique, and the detection is carried out at room temperature. The target probes are previously labeled with a fluorescent material. The hybridization is detected by the fluorescence emitted from the target probes, which react with the capture probes in the gel. Therefore, transparency of the gel at room temperature is a requisite for easy detection of the fluorescence response.

The existing hydrogel-type biochips satisfy requirement (b). However, the diffusivity or permeability of the target probes through the gel network is low, and thus, a long analysis time is required.

In order to alleviate this problem, we proposed a semi-interpenetrating polymer network (semi-IPN) gel, which consists of an amphiphilic gel and an interpenetrating thermosensitive polymer [6]. Thermosensitive polymers are soluble in water below their lower critical solution temperature (LCST) and gel is transparent; however, they are insoluble in water above the LCST. Therefore, on heating, the interpenetrating thermosensitive polymers of the

semi-IPN gel become entangled in the gel network resulting in shrinkage of a part of the gel network without a large volume change, as shown in Fig. 1. This structural change results in enhanced permeability of materials through the gel. This phenomenon was confirmed in our previous study [6]. In that study, the DMAA gel was used as the base gel and poly(*N*-isopropylacrylamide) (poly(NIPAM)) was used as the interpenetrating thermosensitive polymer. The DMAA gel is amphiphilic and its swelling degree is almost independent of temperature and the composition of the solution such as the salt concentration. Poly(NIPAM), on the other hand, is well known as a typical thermosensitive polymer with an LCST of approximately 32°C in water.

There have been several reports on semi-IPN gels composed of a hydrophilic gel and an interpenetrating thermosensitive polymer; these reports state that the responsiveness, mechanical strength, and partition of hydrophobic materials in these gels have been improved by controlling the temperature [7-10]. The thermosensitive properties of these semi-IPN gels have been examined only in water. However, biochips are usually used in buffer solutions, which are composed of a relatively high concentration of salts and a surfactant. Nonetheless, there is a dearth of research of the thermosensitive behavior of the semi-IPN gel composed of an amphiphilic gel and interpenetrating thermosensitive polymer in buffer solutions.

Typical buffer solutions used in biochips are composed of sodium chloride (NaCl), sodium citrate, and sodium dodecyl sulfate (SDS). The composition of the buffer solution is usually expressed in terms of SDS and a mixture of sodium chloride and sodium citrate (SSC). The solution represented as 1 × SSC consists of a mixture of 0.15-mol/*l* sodium chloride and 0.015-mol/*l* sodium citrate. In biochips, various concentrations of SSC up to 6 × SSC (0.9-mol/*l* NaCl and 0.09-mol/*l* sodium citrate) are used as the buffer solution.

In our previous study [6], the thermosensitive behavior of the semi-IPN gel composed of DMAA-*co*-NIPAM gel and interpenetrating poly(NIPAM) was examined in the buffer solution containing 2 × SSC and 0.2% SDS. The LCST of poly(NIPAM) decreases with increase in the concentration of salt, and at high salt concentrations poly(NIPAM) is insoluble at room temperature [11]. Therefore, the semi-IPN gel proposed in our previous study could

not be at SSC concentrations higher than 3 × SSC.

In order to obviate this problem, we paid attention to zwitterionic polymers such as poly(*N*,*N*-dimethyl(acrylamidopropyl)ammonium propane sulfonate) (poly(DMAAPS)), the chemical structure of which is shown in Fig. 2. Poly(DMAAPS) is insoluble in water at lower temperatures, and is soluble at high temperatures; in other words, the poly(DMAAPS) has an upper critical solution temperature (UCST). The zwitterionic polymer is considered to be in a collapsed-coil state in water below the UCST due to the intrachain and/or interchain interactions; however, above the UCST, the polymer adopts an extended conformation because the thermal motion of the polymer molecules overcomes the intrachain and/or interchain interactions [12]. Furthermore, the intrachain and/or interchain interactions are disrupted by the addition of salts, and the resulting expansion of the polymer chain leads to a decreased UCST [12-16]. In other words, the solubility of the polymer is enhanced by dissolved salts. In addition, the LCST of poly(NIPAM) in aqueous salt solutions is increased by copolymerization with the zwitterionic monomer [17-21]. Based on these properties of zwitterionic polymers, it is expected that the LCST of poly(NIPAM) in buffer solutions containing a high concentration of SSC can be controlled by copolymerization with DMAAPS.

In this study, the effects of the preparation conditions of the semi-IPN gel, such as the copolymerization ratio of NIPAM in DMAA gel, the content of poly(NIPAM-*co*-DMAAPS), and the copolymerization ratio of DMAAPS on the thermosensitive behavior of the semi-IPN gel in buffer solutions containing various concentrations of SSC were examined experimentally. The thermosensitive behaviors were examined by measuring the changes in the transmittance through the semi-IPN gel and the changes in the permeability of the buffer solution through the semi-IPN gel membrane with temperature.

The main purpose of this study is to confirm the structural changes in the gel network shown in Fig. 1 in a buffer solution containing salts of relatively high concentrations. To promote the permeation or diffusion of materials through the gel network, the effective pore size of the gel network should be large, or in other words, the cross-linking density should be

small. However, in such a case, the resultant mechanical strength of the gel decreases, and it is difficult to use these gels for biochips. On the other hand, it is considered that the mechanical strength is maintained by interpenetrating polymer in the gel network, even if the cross-linking density is low. In this study, the effect of the concentration of cross-linker in the preparation of the gel on the permeability was not investigated; in other words, the effective pore size of the network of the DMAA-*co*-NIPAM gel was not considered. Our attention is focused on the structural changes in the gel network.

2. Experimental section

2.1. Synthesis

2.1.1. Synthesis of DMAAPS

DMAAPS was synthesized by the ring-opening reaction of 1,3-propanesultone with *N*,*N*-dimethylaminopropylacrylamide (DMAPAA) in the presence of acetonitrile, at 30°C for 1.5 hours [22]. DMAPAA and 1,3-propanesultone were kindly supplied by Kohjin Co. Ltd., and were used without further purification. A mixture of 1,3-propanesultone (75 g) and acetonitrile (75 g) was added dropwise to the mixture of DMAPAA (100 g) and acetonitrile (200 g) under continuous stirring with a magnetic stirrer over a period of 1.5 hours at 30°C, and the mixing was continued for 16 hours at the same temperature. The resulting white precipitate was filtered, washed with acetone, and dried under vacuum.

2.1.2. Synthesis of poly(NIPAM), poly(DMAAPS), and poly(NIPAM-co-DMAAPS)

NIPAM was also kindly supplied by Kohjin Co. Ltd. and was purified by recrystallization from hexane before use. Poly(NIPAM) was prepared by radical polymerization in the same manner as that reported in our previous paper [23]. *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and ammonium peroxodisulfate (APS) were used as an accelerator and an initiator, respectively. The prepared poly(NIPAM) was purified by dialysis over a period of one week,

using the membrane Cellu Step T3, Nominal MWCO: 12000-14000, (Membrane Filtration Products, Inc.). The average molecular weight of poly(NIPAM) was estimated from the intrinsic viscosity as previously reported [23]. Intrinsic viscosity measurement was performed using the polymer solution dissolved in tetrahydrofuran at 27°C, and the following equation that was proposed by Fujishige [24] was used to calculate the number average molecular weight $\overline{M_n}$:

$$[\eta] = 9.59 \times 10^{-3} \overline{M_n}^{0.65} \qquad (1)$$

The $\overline{M_n}$ of poly(NIPAM) prepared in this study was about 7.42×10^6.

Poly(DMAAPS) and poly(NIPAM-*co*-DMAAPS) were also prepared by radical polymerization in the same manner as that mentioned above. In the preparation of poly(NIPAM-*co*-DMAAPS), the copolymerization ratio of DMAAPS was 5, 10, and 20 mol%. The molecular weights of these polymers were not evaluated.

2.1.3. Preparation of semi-IPN gels

The semi-IPN gels were prepared in an aqueous solution containing poly(NIPAM-*co*-DMAAPS) by copolymerization using DMAA as the primary monomer, NIPAM as a *co*-monomer, and *N,N′*-methylenebisacrylamide (MBAA) as a cross-linker. The preparation was performed at 20°C by radical polymerization using TEMED and APS. The synthesis conditions of the semi-IPN gels are listed in Table 1.

In order to measure the transmittance through the semi-IPN gels, plate-type gels of 3 mm thickness were prepared. The synthesis was performed between two glass plates separated by a 3-mm-thick spacer. The gels were cut into dimensions of 10 × 20 mm. Cylindrical gels were also synthesized for use in swelling ratio measurements. The synthesis was performed in a glass tube with an inner diameter of 6 mm. The gels were cut into lengths equal to the diameter.

2.2. Measurements

2.2.1. Measurement of transmittance through the buffer solution containing the polymer and through the semi-IPN gel immersed in the buffer solution

The transition behavior of poly(DMAAPS), poly(NIPAM), and poly(NIPAM-*co*-DMAAPS) was investigated by measuring the change in the transmittance as a function of temperature in an aqueous solution containing SSC and SDS, which constitute the buffer solution. In the hydrophilic state of the polymer, the solution is transparent because, in this state, the polymer is soluble in the solution. However, when the change to the hydrophobic state is induced by heating or cooling, the solution becomes opaque or milky white, because the hydrophobic polymer is insoluble in the aqueous solution. Therefore, the transition temperature (LCST or UCST) can be estimated by measuring the change in the transmittance through the solution as a function of temperature. The transmittance was measured at 600 nm using a spectrophotometer equipped with a temperature-control system (V-530, Japan Spectroscopy Co., Ltd.) and the measurements were performed for the solutions with various concentrations of NaCl, sodium citrate, and SDS.

The temperature dependence of the transmittance through the semi-IPN gels immersed in the buffer solution was also measured in order to examine their thermosensitive behaviors in the buffer solution. The measurements were performed at 600 nm using the aforementioned spectrophotometer. Measurements were carried out using a quartz cell with a path length of 10 mm, and the gel was placed perpendicular to the light path and gently pressed on the inner wall of the quartz cell filled with the buffer solution.

2.2.2. Measurement of the swelling properties of semi-IPN gels

The effect of the temperature on the degree of swelling of the semi-IPN gels in the buffer solution was examined using the cylindrical gels. The dried gels were immersed in the buffer solution at 25°C until the swelling of the gel reached equilibrium, and the swelling diameter of the gel was measured with a cathetometer. The temperature was then increased to the desired temperature, and the equilibrium swelling diameter was measured again. This

procedure was repeated up to 70°C.

2.2.3. Measurement of the temperature dependence of the permeability of the buffer solution through the semi-IPN gel membrane

The semi-IPN gel membrane was synthesized in the hole of the gel holder composed of three glass plates with a total thickness of 3.6 mm as shown in Fig. 3(a). The diameter of the hole of the center plate was 30 mm, and a cut was made in a part of the center plate to introduce the reactant solution. The diameter of the holes on the glass plates on both sides was 20 mm. The surface of the glass plate containing holes was treated with chlorodimethylvinylsilane to introduce double bonds on the wall surface [25]. The synthesis of the gel was performed by radical polymerization. The preparation procedure was as follows. The hole was covered by two slide glasses, and the reactant solution, which was previously degassed, was poured into the hole through a cut in the center plate. The reactant solution was composed of two aqueous solutions. The first was the monomer solution containing DMAA, NIPAM, MBAA, TEMED, and poly(NIPAM), and the other was the APS solution. After completion of the polymerization of the gel, the slide glasses covering the holes were removed, and the surface of the gel membrane was reinforced by a stainless steel net, which was attached to the glass plate by quick-drying glue as shown in Fig. 3(a). Then, the membrane was washed by immersing it into distilled water to remove unreacted monomers and other reagents. The DMAA-*co*-NIPAM gel membrane without poly(NIPAM-*co*-DMAAPS) was also prepared for comparison. The gel was prepared using the following composition: DMAA/NIPAM/MBAA: 700/300/4 mol/m^3 (NP30). The poly(NIPAM-*co*-DMAAPS) concentration was 0.5 wt%.

The permeability of the buffer solution through the gel membrane was measured with the apparatus shown in Fig. 3(b). The apparatus was composed of two permeation cells made of glass, a 500 mm long glass column was used to generate the hydrostatic pressure and a calibrated glass pipette was used to measure the flow rate. The gel holder shown Fig. 3(a) was placed and fixed between two permeation cells. The apparatus was immersed in the water

bath. Then, the buffer solution was poured into both cells, and the apparatus was left overnight to reach an equilibrium swelling state and the steady permeation rate at 35°C, after which the position of the meniscus in the pipette was measured as a function of time. Then, the temperature was raised to the desired temperature and the apparatus was again left overnight, and the measurement was repeated. The procedure was repeated until the temperature reached 50°C.

The permeation coefficient *K* was estimated by the following equation:

$$v = \frac{V}{A} = K \cdot \frac{\Delta P}{d \cdot \mu} \qquad (2)$$

where v is the velocity of the buffer solution; *A* is the cross-sectional area of the gel membrane; ΔP is the pressure applied to the gel membrane; *d* is the thickness of the gel membrane and μ is the viscosity of the buffer solution.

3. Results and discussion

3.1. Thermosensitive behavior of poly(DMAAPS) in water

Fig. 4 shows the temperature dependence of the transmittance through the aqueous solution of poly(DMAAPS). At lower temperatures, the solution was opaque or milky white, and the transmittance was very low. Increasing the temperature resulted in a change in the solution from opaque to completely transparent, and the transmittance increased to near 100%. In other words, the poly(DMAAPS) has an UCST. Below the UCST, the zwitterionic polymer forms aggregates because the intrachain and/or interchain interactions between the side chains of the polymer are larger than the force of molecular motion, whereas above the UCST, the polymer is soluble in water because thermal motion becomes larger than the interactions between the side chains of the polymer [11]. As the zwitterionic polymer concentration was increased, there was an increase in the UCST. When the polymer concentration increase, the intrachain and/or interchain parings also increase, and more

thermal energy is required to overcome the intrachain and/or interchain interactions. Therefore, the UCST also increases. On the other hand, when the polymer concentration was low, the change in the transmittance was broad. This is considered due to the fact that the interchain and/or intrachain parings decreased and are distributed inhomogeneously.

3.2. Thermosensitive behavior of poly(NIPAM), poly(DMAAPS), and poly(NIPAM-co-DMAAPS) in the buffer solution

Fig. 5 shows the effect of the concentration of NaCl in the solution containing the poly(DMAAPS) on the transmittance of the solution. The concentration of poly(DMAAPS) and the measurement temperature were fixed at 10 g/*l* and 25°C, respectively. The transmittance increased sharply at approximately 1.2 g/*l* of NaCl. This observation implies that the zwitterionic polymer may be soluble in the solution at a high NaCl concentration.

Poly(NIPAM), on the other hand, is a representative polymer with a LCST of approximately 32°C in water, and it is well known that the LCST decreases as the concentration of the salt is increased [11]. Fig. 6 shows the effect of NaCl concentration in the solution on the transmittance of the solution containing poly(NIPAM). These data were obtained in this study. From the figure it can be seen that the LCST decreases with increase in NaCl concentration. These phenomena are attributed to the fact that the poly(NIPAM) molecules become more hydrophobic in salt solutions because the salt molecules destroy the hydration layer around the poly(NIPAM) molecules. Therefore, poly(NIPAM) is insoluble in solutions having a high salt concentration at room temperature.

It is expected that the thermosensitive properties of poly(NIPAM) in salt solution can be easily changed by copolymerizing DMAAPS with NIPAM. Fig. 7 shows the effect of NaCl concentration in the solution containing poly(NIPAM-*co*-DMAAPS) on the LCST. The copolymerization ratio of DMAAPS was fixed at 10 mol%. From the figure it can be seen that the LCST of poly(NIPAM-*co*-DMAAPS) increased compared with that of poly(NIPAM) at the same NaCl concentration. This result is thought to be due to the fact that the interaction

between the zwitterionic polymer and salt inhibits the dehydration of poly(NIPAM).

Fig. 8 shows the temperature dependence of the transmittance of the poly(NIPAM) (Fig. 8(a)) and poly(NIPAM-*co*-DMAAPS) (Fig. 8(b)) solutions containing various concentrations of sodium citrate. The LCST of poly(NIPAM) decreased as the concentration of sodium citrate increased; however, copolymerization with DMAAPS results in an increase of the LCST compared with that of poly(NIPAM) in addition to the effect of the NaCl concentration as shown in Fig. 7. In Figs. 7 and 8, it can be seen that the effect of the salt on the LCST of the poly(NIPAM-*co*-DMAAPS) is larger than that on the LCST of the poly(NIPAM). This reason is attribute to the fact that LCST of an ionic thermosensitive polymer is largely affected by the ions in the solution or, in other words, by the salt in the solution.

Fig. 9 shows the temperature dependence of the transmittance of the poly(NIPAM) (Fig. 9(a)) and poly(NIPAM-*co*-DMAAPS) (Fig. 9(b)) solutions containing various concentrations of SDS. The LCST of poly(NIPAM) increased as the concentration of SDS was increased. Furthermore, the decrease in the transmittance became less as the concentration of SDS increased. These results are different from those in the solution containing NaCl or sodium citrate. These phenomena are well known and explained by the interaction between the hydrophobic groups of SDS and the isopropyl groups of poly(NIPAM) [26-28]. A similar tendency was observed for the solution containing the poly(NIPAM-*co*-DMAAPS); however, the LCST of poly(NIPAM-*co*-DMAAPS) was higher than that of the poly(NIPAM) solution at a comparable SDS concentration.

Fig. 10 shows the effect of the copolymerization ratio of DMAAPS on the transition behavior of poly(NIPAM-*co*-DMAAPS) in water (Fig. 10(a)) and in the buffer solutions composed of mixtures of SSC and SDS (Figs. 10(b), 10(c), and 10(d)). The SDS concentration was fixed at 0.2 wt% and the SSC concentrations were 2 ×, 4 ×, and 6 × SSC. The solutions represented as 2 × SSC, 4 × SSC and 6 × SSC consist of a mixture of 0.30-mol/*l* NaCl and 0.030-mol/*l* sodium citrate, 0.60-mol/*l* NaCl and 0.060-mol/*l* sodium citrate and 0.90-mol/*l* NaCl and 0.090-mol/*l* sodium citrate, respectively. In water, the LCST increased as the copolymerization ratio of DMAAPS increased, and the sharpness of the transition was lost.

In the buffer solution containing 2 × SSC (Fig. 10(b)), the transition of poly(NIPAM) was broad, and the LCSTs of the poly(NIPAM-*co*-DMAAPS) with varying copolymerization ratios increased as the copolymerization ratio of DMAAPS increased; however, the transitions were not clear. These results are attributed to the fact that the interaction of poly(NIPAM) and SDS, which inhibits the transition of poly(NIPAM), is stronger than that of poly(NIPAM) and SSC, which promotes the transition of poly(NIPAM). In the buffer solution containing 4 × SSC (Fig. 10(c)), poly(NIPAM) did not dissolve. The LCSTs of poly(NIPAM-*co*-DMAAPS) in the buffer solution containing 4 × SSC were lower than those in the buffer solution containing 2 × SSC. Furthermore, in the buffer solution containing 6 × SSC (Fig. 10(d)), the poly(NIPAM-*co*-DMAAPS) copolymerized using 5 mol% DMAAPS also did not dissolve at room temperature, and therefore the result was not shown; the transition of the polymer copolymerized using 10 mol% DMAAPS was observed between 30–40°C; and the transition of the polymer copolymerized using 20 mol% DMAAPS was not observed in the experimental temperature range. These results imply that the transition of poly(NIPAM) in the buffer solution composed of various concentrations of salt can be controlled by copolymerization with the zwitterionic DMAAPS at various ratios.

The change in the transmittance, as the temperature was decreased, was also measured in the solution of 4 × SSC using poly(NIPAM-*co*-DMAAPS) copolymerized 10 mol% DMAAPS. Hysteresis was observed. However, the temperature difference was small. The curve of the transmittance shifted to lower temperature of approximately 3°C as compared with that through the upward process.

3.3. Stability of semi-IPN gels composed of DMAA-co-NIPAM gel and interpenetrating poly(NIPAM-co-DMAAPS).

Fig. 11 shows the change in the transmittance through the semi-IPN gels with time. Three kinds of gels were used: NP10, NP20 and NP30, which were prepared in the poly(NIPAM-*co*-DMAAPS) solution of 0.5 wt%. The semi-IPN gels were immersed in a

buffer solution composed of 6 × SSC and 0.2% SDS at 30°C, and the transmittance through the semi-IPN gels was measured at 50°C and at the desired time interval. The initial transmittance through the semi-IPN gels decreased as the copolymerization ratio of NIPAM in the gel network was increased, and was almost 0% for the NP30 semi-IPN gel, which was opaque or milky white.

It is well known that the network distribution of the transparent gel is homogeneous macroscopically, whereas that of the opaque gel consists of dense and coarse networks, in other words, the structure is inhomogeneous [29]. The formation of the inhomogeneous network structure can be attributed to the fact that the interpenetrating poly(NIPAM-*co*-DMAAPS) molecules become entangled in the gel network resulting in shrinkage of a part of the gel network, as shown in Fig. 1.

The transmittance through the NP10 semi-IPN gel increased slightly with time. This increase in transmittance implies that there was outflow of a part of the interpenetrating poly(NIPAM-*co*-DMAAPS) molecules in the semi-IPN gel from the gel. On the other hand, the transmittances through the NP20 and NP30 semi-IPN gels remained constant during the course of the experiment, which implies that the interpenetrating poly(NIPAM-*co*-DMAAPS) molecules were held stably in the gel network. This stability was attributed to the fact that the interaction between the poly(NIPAM) molecules and the NIPAM components in the gel network suppresses the outflow of the interpenetrating polymer molecules from the gel.

3.4. Thermosensitive properties of the semi-IPN gel in the buffer solution

Fig. 12 shows the effect of the content of the interpenetrating poly(NIPAM-*co*-DMAAPS) in the semi-IPN gel on the thermosensitive properties of the semi-IPN gel in the buffer solution, which is evaluated by the change in the transmittance through the semi-IPN gel with temperature. The NP20 gel was used. The copolymerization ratio of DMAAPS in the interpenetrating poly(NIPAM-*co*-DMAAPS) was 10 mol%. The measurement of the transmittance was performed in the buffer solution comprised of 6 × SSC and 0.2% SDS. The

transmittance through the gel without the interpenetrating polymer did not decrease in the experimental temperature range; in other words, the network structure of the gel did not change. The transmittance through the semi-IPN gel containing 0.5 wt% interpenetrating poly(NIPAM-*co*-DMAAPS) began to decrease at about 30°C and reached 20% at 50°C. The transition behavior of this semi-IPN gel was similar to that of poly(NIPAM-*co*-DMAAPS) shown in Fig. 10(d). Based on these results, it is suggested that the gel network changed from a homogeneous structure to an inhomogeneous structure composed of the coarse and dense networks because of the transition of the interpenetrating poly(NIPAM-*co*-DMAAPS). On the other hand, the transmittance through the semi-IPN gel containing 1.0 wt% interpenetrating poly(NIPAM-*co*-DMAAPS) was low even at 30°C. These results suggest that in the presence of a higher content of interpenetrating poly(NIPAM-*co*-DMAAPS), the inhomogeneous network structure forms in the preparation of the semi-IPN gel.

3.5. Swelling properties of the semi-IPN gels in the buffer solution

Fig. 13 shows the temperature dependence of the swelling diameter of the cylindrical semi-IPN gels in the buffer solution comprised of 6 × SSC and 0.2% SDS. The NP10, NP20, and NP30 gels were used, and the content of poly(NIPAM-*co*-DMAAPS) was fixed at 0.5 wt%. The gel diameter decreased slightly with increasing temperature. Furthermore, the change in the gel diameter was dependent on the copolymerization ratio of NIPAM in the gel network, and decreased as the copolymerization ratio of NIPAM was increased. On the other hand, the gel diameter was hardly affected by the content of the interpenetrating poly(NIPAM-*co*-DMAAPS) in the content range of 0–0.5 wt%. These results indicate that the change in the network structure of the semi-IPN gel occurred without a large volume change. The change in the gel size observed here is sufficiently small to enable the use of the semi-IPN gel as a base gel for biochips.

3.6. Temperature dependence of the permeability of the buffer solution through the semi-IPN

gel

Fig. 14 shows the temperature dependence of the permeability of the buffer solution containing 6 × SSC and 0.2% SDS through the semi-IPN gel membrane. The NP30 semi-IPN gel prepared in the 0.5 wt% poly(NIPAM-*co*-DMAAPS) solution was used. The copolymerization ratio of DMAAPS in the interpenetrating poly(NIPAM-*co*-DMAAPS) was 10 mol%. The results for the gel membrane without poly(NIPAM-*co*-DMAAPS) are also shown for comparison. The permeability of the buffer solution through the gel membrane without poly(NIPAM-*co*-DMAAPS) decreased slightly as the temperature increased. The decrease in permeability with increasing temperature can be attributed to the fact that the volume of the gel decreased slightly as the temperature increased; in other words, the effective pore size of the gel network decreased slightly. On the other hand, the permeability through the semi-IPN gel increased drastically between 35 and 45°C. This temperature range corresponds to the transition behavior of the poly(NIPAM-*co*-DMAAPS) shown in Fig. 10(d) and the semi-IPN gel shown in Fig. 12. It is considered that the buffer solution could permeate easily through the coarse network. On the basis of these results, the structural change of the semi-IPN gel network due to the transition of the interpenetrating poly(NIPAM-*co*-DMAAPS) was confirmed.

4. Conclusion

The transition behaviors of the poly(NIPAM-*co*-DMAAPS) and the semi-IPN gel composed of the DMAA-*co*-NIPAM gel and the interpenetrating thermosensitive poly(NIPAM-*co*-DMAAPS) were examined in buffer solutions containing relatively high concentrations of SSC. The LCST of poly(NIPAM-*co*-DMAAPS), in which zwitterionic DMAAPS is copolymerized with NIPAM, increased as the polymerization ratio of DMAAPS increased and decreased with increasing SSC concentration. The LCST of poly(NIPAM-*co*-DMAAPS) could be controlled by selecting the polymerization ratio of

DMAAPS in the desired temperature range even at a SSC concentration of 6 × SSC, which is a quite high salt concentration.

The change in the semi-IPN gel from transparent to opaque or milky white corresponding to the transition of poly(NIPAM-*co*-DMAAPS) occurred without a large volume change. The development of opacity implies that the gel network changed from a homogeneous structure to an inhomogeneous structure composed of the coarse and dense networks due to the transition of the interpenetrating poly(NIPAM-*co*-DMAAPS). This structural change in the network was confirmed by the fact that the permeability of the buffer solution through the gel membrane increased drastically in the temperature range corresponding to the transition of the poly(NIPAM-*co*-DMAAPS) and the semi-IPN gel.

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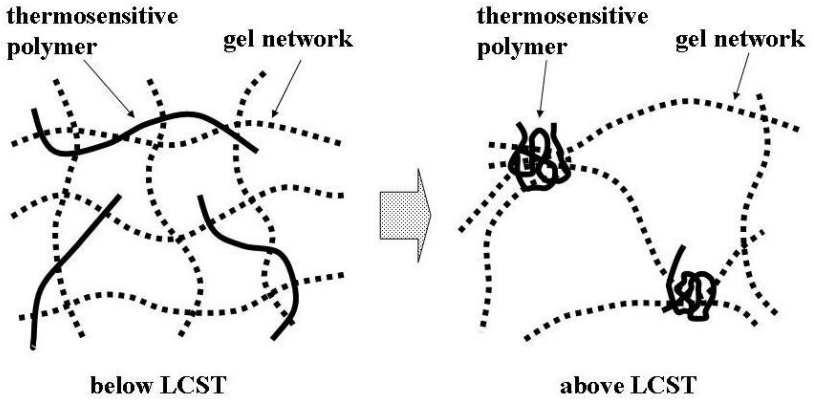

Fig. 1 Conceptual representation of structural changes in the semi-IPN gel networks.

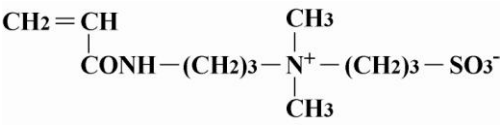

Fig. 2 Chemical structure of DMAAPS.

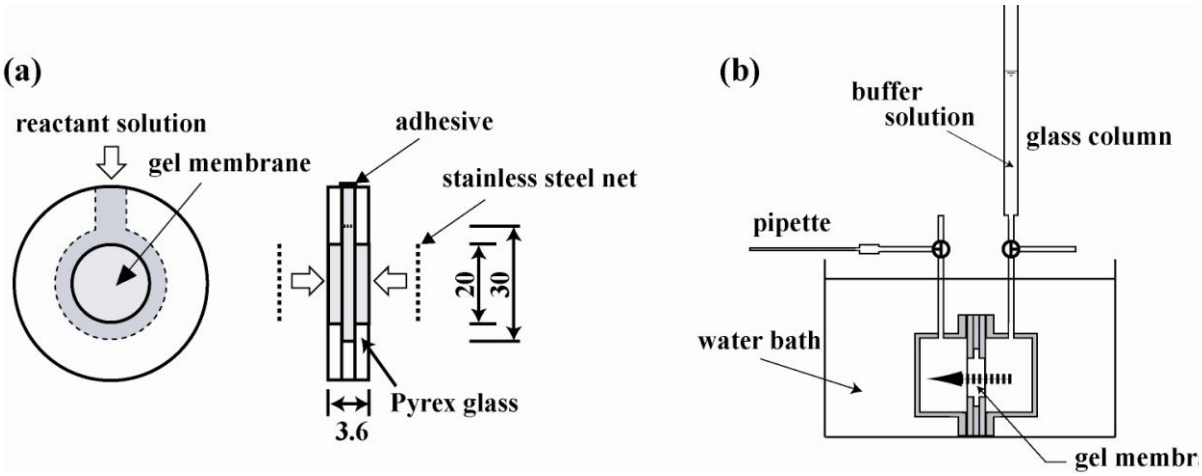

Fig. 3 (a) Gel holder and **(b)** apparatus for permeability measurements.

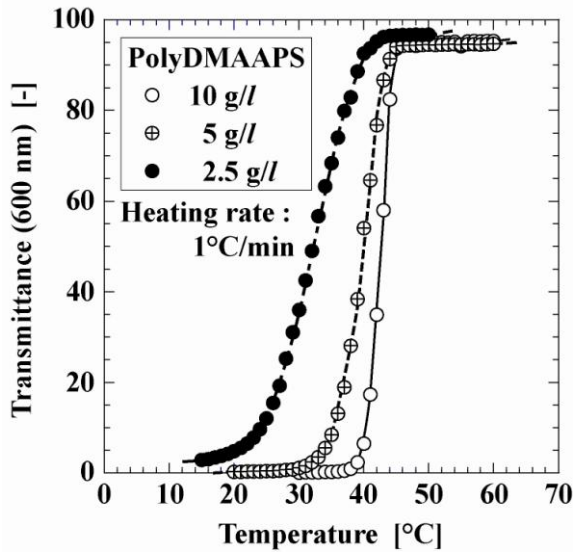

Fig. 4 Transition behavior of poly(DMAAPS) in water.

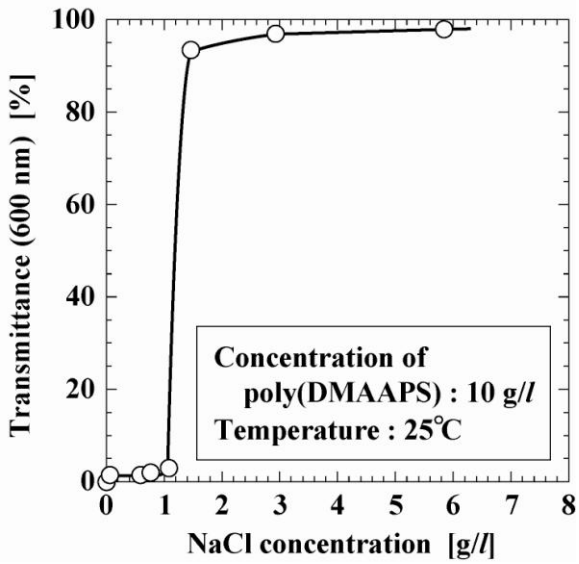

Fig. 5 Transition behavior of poly(DMAAPS) in sodium chloride (NaCl) solution.

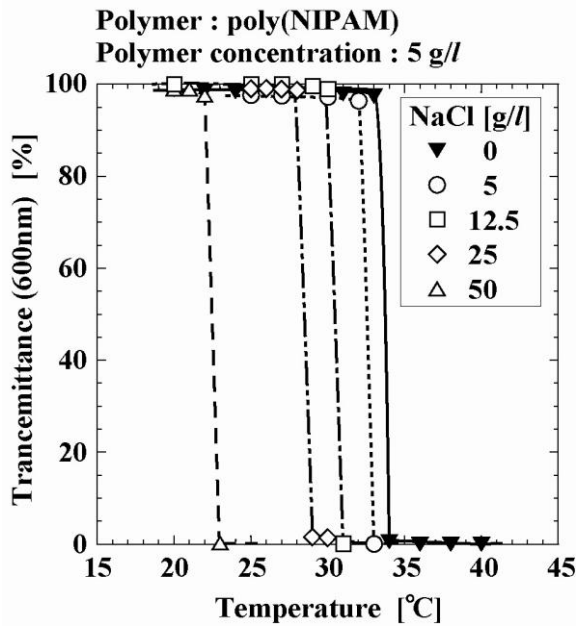

Fig. 6 Transition behavior of poly(NIPAM) in sodium chloride (NaCl) solution.

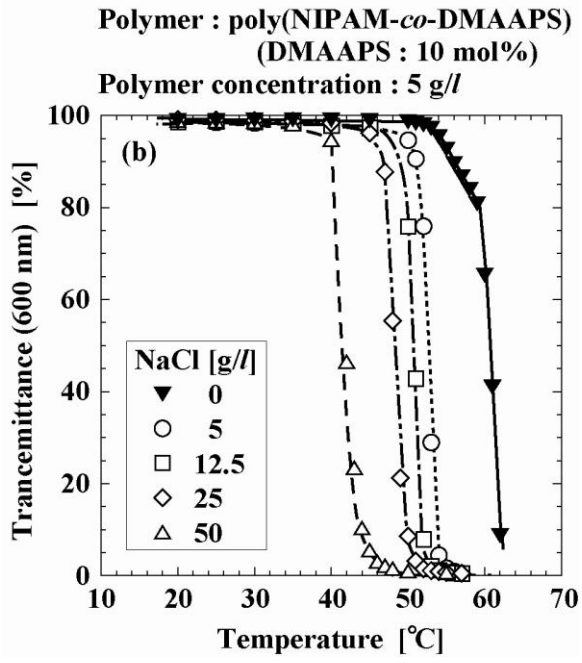

Fig. 7 Transition behavior of poly(NIPAM-*co*-DMAAPS) in sodium chloride (NaCl) solution.

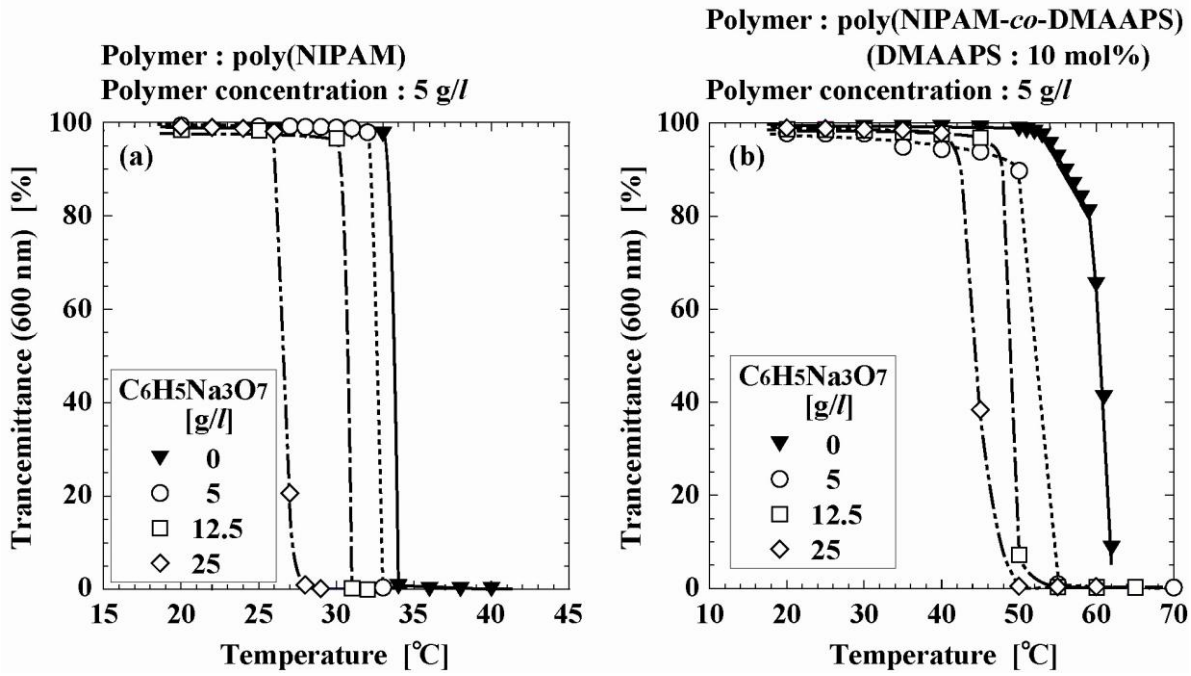

Fig. 8 Transition behavior of (a) poly(NIPAM) (b) poly(NIPAM-*co*-DMAAPS) in sodium citrate solution.

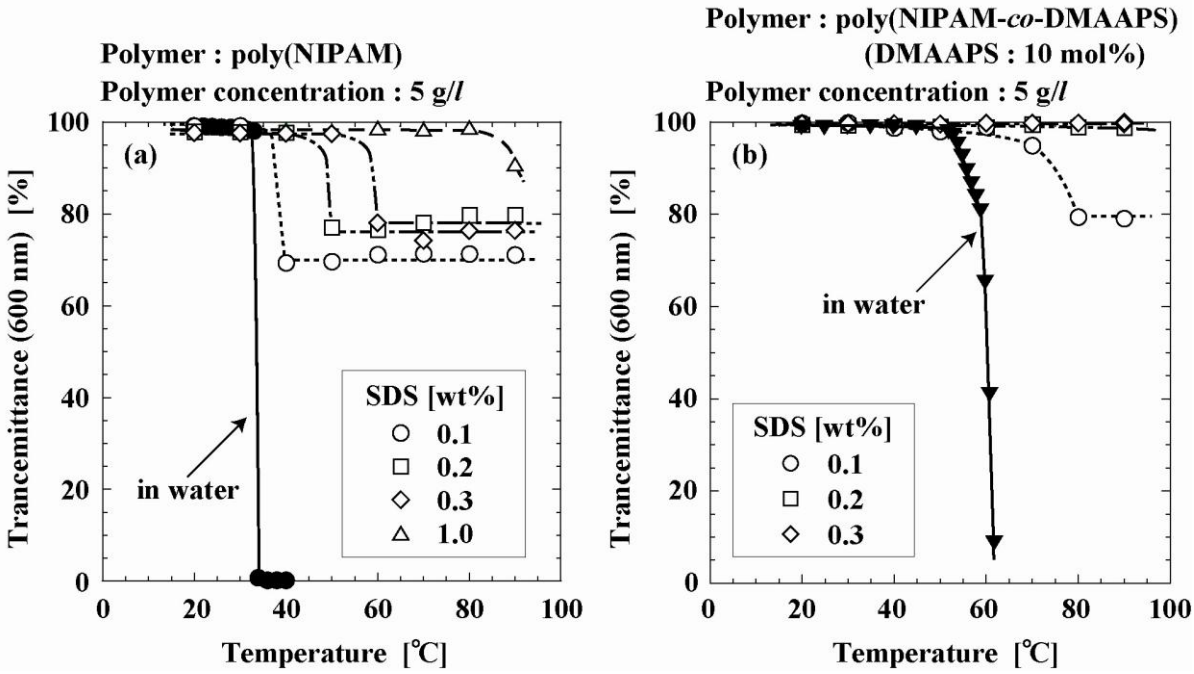

Fig. 9 Transition behavior of (a) poly(NIPAM) (b) poly(NIPAM-*co*-DMAAPS) in sodium dodecyl sulfate (SDS) solution.

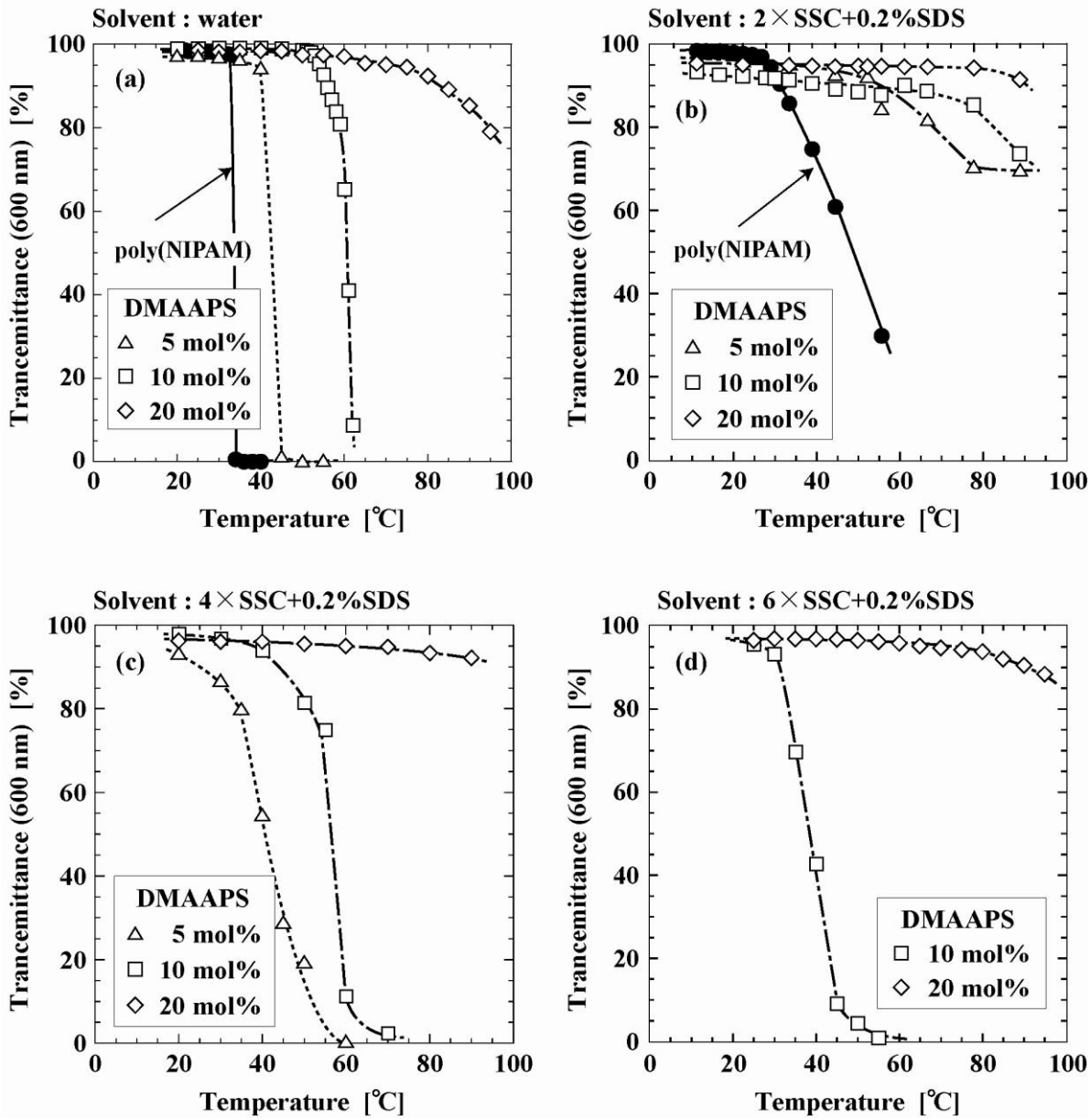

Fig. 10 Transition behavior of poly(NIPAM-*co*-DMAAPS) in buffer solution consisting of SSC and SDS. The SDS concentration was fixed at 0.2 wt%, while that of SSC was varied from 2 × SSC to 6 × SSC. The polymerization ratio of DMAAPS was varied from 0–20 mol%. The concentration of poly(NIPAM-*co*-DMAAPS) was 0.5 wt%.

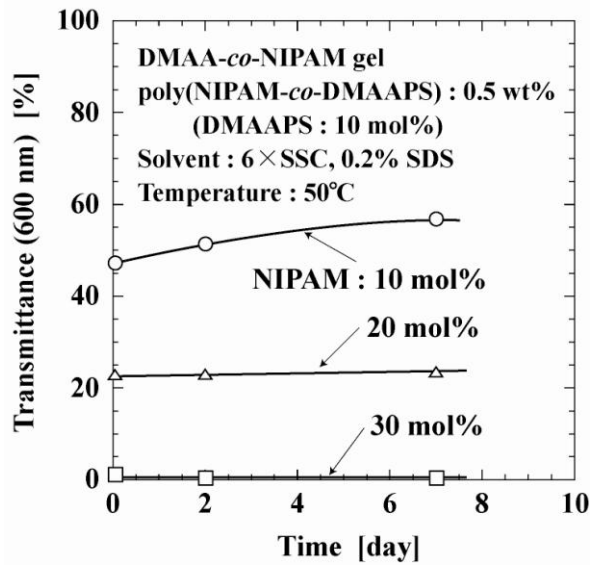

Fig. 11 The transmittance change through the semi-IPN gels with time. The gel was prepared with various copolymerization ratios of NIPAM. The polymerization ratio of DMAAPS is 10 mol%. The concentration of poly(NIPAM-*co*-DMAAPS) is 0.5 wt%.

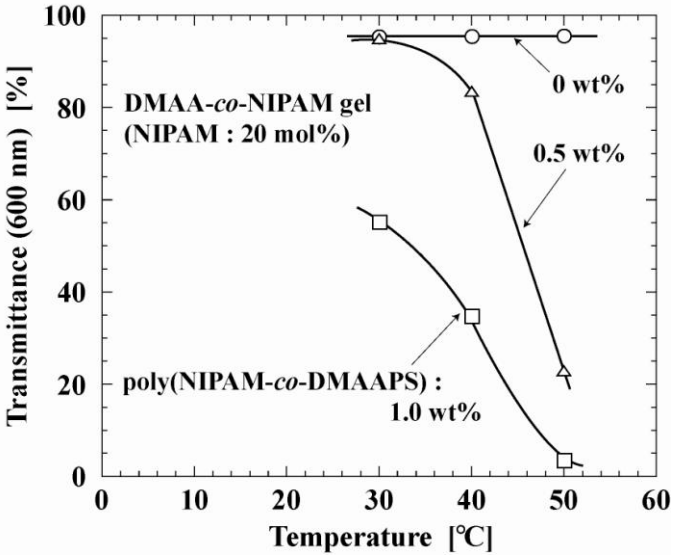

Fig. 12 Temperature dependence of the transmittance through NP20 semi-IPN gels. The gel was prepared in the solution containing various concentrations of poly(NIPAM-*co*-DMAAPS).

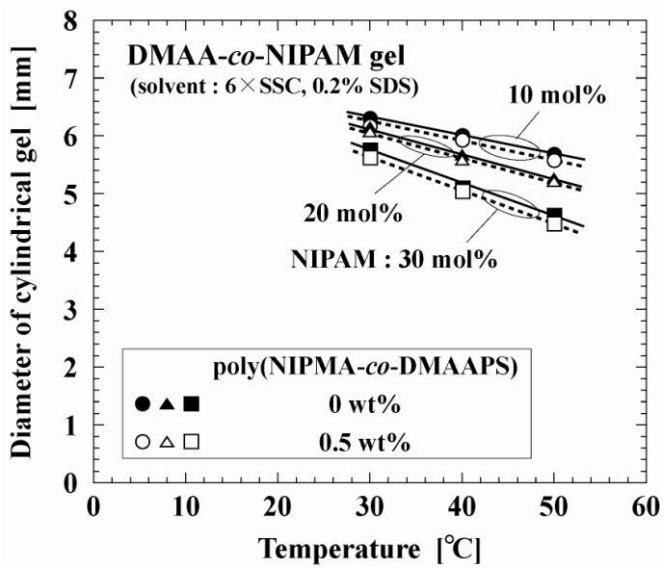

Fig. 13 Effect of the copolymerization ratio of NIPAM in the gel network on the temperature dependence of the swelling diameter of the semi-IPN gel.

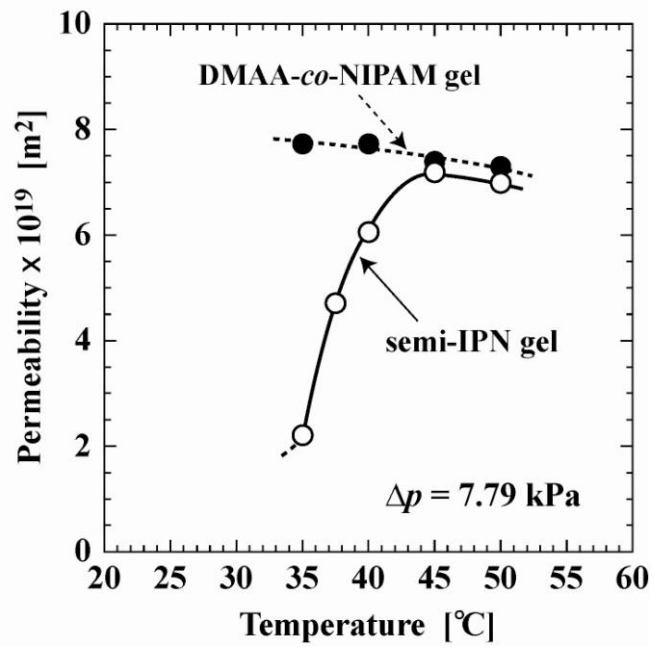

Fig. 14 Temperature dependence of the permeability of the buffer solution through NP30 gel without poly(NIPAM-*co*-DMAAPS) and NP20 semi-IPN gel.

Table 1

Synthesis conditions of semi-IPN gels

[mol/m^3]

		NP0	NP10	NP20	NP30
Monomer	: *N*,*N*-dimethylacrylamide (DMAA)	1000	900	800	700
Co-monomer	: *N*-isopropylacrylamide (NIPAM)	0	100	200	300
	(Copolymerization ratio of NIPAM)	0 mol%	10 mol%	20 mol%	30 mol%)
Linker	: *N*,*N*′-methylenebisacrylamide (MBAA)		4		
Accelerator	: *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED)		10		
Initiator	: ammonium peroxodisulfate (APS)		0.5		

Solvent: aqueous poly(NIPAM-*co*-DMAAPS) solution: 0.1, 0.3, 0.5 wt%
Copolymerization ratio of DMAAPS: 10 mol%
Temperature: 20°C
Reaction time: 6 h